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(54) Title: HYDROXYAPATITE FORMING DRY PARTICULATE AGGLOMERATE AND METHODS THEREFOR

(57) Abstract

The present invention is directed to a particulate and a method for its production. The present invention is also directed to a synthetic bone-like HAp composition produced from said particulate agglomerate composition and, optionally, a polymeric material capable of promoting mineralization of hydroxyapatite, which is useful for fixing prosthetic devices, useful as bone substitutes to directly fill bone defects, to provide substrates for cartilage, and to repair teeth, and methods of making such preparations.

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# HYDROXYAPATITE FORMING DRY PARTICULATE AGGLOMERATE AND METHODS THEREFOR

**Government Sponsorship**

This invention was made with Government support under Grant No. DE 09421 awarded by the NIH. The Government has certain rights in the invention.

## BACKGROUND OF THE INVENTION

Field of the Invention

The present invention is directed to a dry particulate agglomerate composition capable of producing phase-pure hydroxyapatite without additional sources of calcium and/or phosphate in the presence of an aqueous liquid consisting essentially of agglomerates, each individual agglomerate being a homogenous mixture of (i) $CaHPO_4 \cdot 2H_2O$ particles and (ii) particles of a calcium source comprising $Ca_4(PO_4)_2O$ wherein said $CaHPO_4 \cdot 2H_2O$ and said calcium source comprising $Ca_4(PO_4)_2O$ are present in an amount sufficient to form phase-pure HAp having a Ca/P ratio of 1.5 to 1.67 and a method for its production. The dry particulate agglomerate composition

of the present invention is capable of forming phase-pure hydroxyapatite *in vivo* at physiological temperatures within 1 hour in the presence of an aqueous liquid. The present invention is also directed to making a synthetic bone-like or dental composition comprising said particulate agglomerate composition and optionally, a polymeric material capable of promoting mineralization of hydroxyapatite, which are useful for fixing prosthetic devices, useful as bone substitutes to directly fill bone defects, to provide substrates for cartilage, and to repair teeth, and methods of making such preparations.

Description of Related Art

The microstructural features of teeth and bone and the processes by which remodeling occurs are of importance in determining desirable microstructures in bone-like substitutes. Because bone substitutes must emulate the function of bone, bone must bond well with them. The nature of this bonding will depend on the pore sizes in the polymeric material capable of forming mineralization of hydroxyapatite. It has been shown that the response of bone to porous implants depends on the pore size. Bone growth occurs in close apposition to an implant which contain 40 μm pores regardless of whether the implant is a metallic or a ceramic material. For pore sizes of 100 μm, bone will fill in the pore and for pores sizes in excess of approximately 150 μm Haversion systems will develop and facilitate the formation of a strong bond with the bone substitute. The ability to directly control microstructural development in a prosthesis composed of a resorbable material and consequently to control its porosity has a major influence on bone intergrowth and prosthesis remodeling. Unfortunately, mechanical integrity and porosity are conflicting functional requirements. Thus,

it is unlikely that a single material would be capable of meeting the function requirements of bone. Indeed, none has been found. The alternative is to develop a composite which would exhibit adequate mechanical properties along with the porosity needed to meet the biological requirements for remodeling.

There have been many attempts to prepare a substitute bone material and there are numerous patents disclosing methods of preparation of such substitute bone materials. U.S. Patent No. 4,440,750 to Glowacki, *et al.* discloses a plastic dispersion of demineralized bone powder and reconstituted native atelopeptide collagen fibers in a continuous aqueous phase to repair or construct bone by injecting or implanting it at the repair or constructive site. U.S. Patent No. 4,516,276 to Mittelmeier employs collagen as a fleece described as a grid or network which is than dusted with apatite powder or granules, or the fiber material is mixed with mineral before being formed into layers for implantation into bone. Some patents claim the use of porous hydroxyapatite, for example U.S. Patent No. 4,629,464 discloses a method for preparation of artificial bone material where sintered microporous hydroxyapatite is used either in a granular or slurried form in a physiological saline solution or in a form of a shaped prosthetic bone substitute. The prepared bone substitute is characterized by an open porous structure. Such an open porous structure allows for ingrowth of natural tissue. Another method and composition of material promoting the growth of bone is disclosed in U.S. Patent No. 5,073,114. The bone growing composition includes two sizes of hydroxyapatite for supporting the growth of the bone, tetracycline for its antibiotic effect, freeze-dried decalcified human bone for promoting bone growth and fibronectin for promoting connective tissue generation and for gelling the bone growing composition. U.S. Patent No. 4,776,890

introduces a process for obtaining a matrix of mineral particles in reconstituted atelopeptide collagen comprising reconstituting a mixture of mineral particles with collagen in solution. However, none of the above discussed bone substitutes undergo reaction *in vivo* which would lead to their exhibiting mechanical properties comparable to those of natural bone.

U.S. Patent Nos. 4,880,610; 5,047,031; and 5,053,212 to Constantz disclose calcium phosphate compositions useful as cements. The process involves merely mixing two dry ingredients, i.e., a calcium source and a phosphoric acid source under reacting conditions to form a kneadable mixture. None of these patents disclose or suggest a dry particulate agglomerate composition, capable of forming phase-pure hydroxyapatite without additional sources of calcium and/or phosphate in the presence of an aqueous liquid, prepared by mixing $CaHPO_4 \cdot 2H_2O$ (brushite) particles and particles of a calcium source comprising $Ca_4(PO_4)_2O$ in a non-aqueous liquid which will not allow the particles to react with each other, and then removing the non-aqueous liquid according to the present invention, and which can be stored in a dry state and which are stable over long periods of time.

In U.S. Patent 5,053,212, water is liberated in the mechanical mixing of the calcium source with the acidic phosphate source under reacting conditions. Disadvantageously, this liberation of water is free to combine with the calcium and acidic phosphate sources which results in calcium phosphates which are slow to react to form HAp, and upon reaction to form HAp again release water, thereby increasing the porosity and limiting the mechanical strength of the resultant product.

W. Brown and Chow, U.S. Patent 4,612,053, disclose mechanically mixing $Ca_4(PO_4)_2O$ with at least one other calcium phosphate selected from the group consisting of

$CaHPO_4.2H_2O$, $CaHPO_4$, $Ca_8H_2(PO_4)_4.5H_2O$ (i.e., $Ca_8(HPO_4)_2(PO_4)_4.5H_2O$), $\alpha$-$Ca_3(PO_4)_2$, $\beta$-$Ca_3(PO_4)_2$ and modified $Ca_3(PO_4)_2$ for use in a dental restorative powder, paste and/or slurry. This mechanical mixing does not result in a dry particulate agglomerate composition wherein each agglomerate is a homogenous mixture of $Ca_4(PO_4)_2O$ and the recited calcium phosphates which would allow phase pure HAp to form within an hour. Moreover, in this patent activation to form HAp occurs by the addition of phosphoric acid which results in a pH below physiologically acceptable levels. Thus, this patent does not disclose or suggest the use of, or the inventive dry particulate agglomerate composition capable of forming hydroxyapatite without additional sources of calcium and/or phosphate, and which said particles can be stored in a dry state for long periods of time and which when mixed with a physiologically acceptable aqueous liquid, can completely react to form phase-pure hydroxyapatite *in vivo* within a surgically relevant time of less than 1 hour, particularly less than 30 minutes, as in the present invention.

The above discussed patents to W. Brown and Chow and Constanz rely on grinding mixtures of particles to a high fineness, which particles in admixture are then reacted in an aqueous solution to form hydroxyapatite.

Neither U.S. Patent 4,612,053, nor U.S. Patents 4,880,610, 5,047,031 or 5,053,212 disclose the preparation of a dry particulate agglomerate composition wherein each agglomerate is a homogenous mixture of $CaHPO_4{\cdot}2H_2O$ particles and particles of a calcium source comprising $Ca_4(PO_4)_2O$ capable of forming phase pure hydroxyapatite wherein the particulate agglomerates are formed as a result of mixing $CaHPO_4{\cdot}2H_2O$ particles and particles of a calcium source comprising $Ca_4(PO_4)_2O$ to a degree of homogeneity which can only be attained when said mixing occurs in a non-aqueous liquid and utilizing

the same in preparing hydroxyapatite compositions useful as a bone-like substitute or a dental composition as in the present invention.

The present invention is directed to a method of preparation of the particulate agglomerate composition, wherein each particulate agglomerate is an intimate mixture of $CaHPO_4 \cdot 2H_2O$ particles and a calcium source comprising $Ca_4(PO_4)_2O$ which are capable of forming phase-pure hydroxyapatite and which can be stored over a long period of time which provided phase-pure HAp, besides exhibiting biocompatibility and the mechanical characteristics required for teeth or artificial bone, can be formed in a surgically relevant time, e.g., usually under 1 hour, particularly under 30 minutes.

Most of the prior art requires prosthesis preparation with cured hydroxyapatite in advance of surgery. This complicates treatment and requires taking measurements and separately fitting of the hydroxyapatite implant. The present invention introduces an novel way of preparing phase pure hydroxyapatite at physiological temperature in a surgically relevant time, i.e., hydroxyapatite formation is complete within 1 hour, usually within 30 minutes.

The advantages of using the dry particulate agglomerate composition of the present invention to make hydroxyapatite as a bone substitute are that the properties can be tailored to serve a variety of needs. These include development of the microstructures of both cancellous (porous) and cortical (dense) bone. In addition, the proportions of the constituent ingredients can be adjusted accordingly as a means of controlling the rate and extent of remodeling. Remodeling is the term used to describe the process of bone replacement. Remodeling in synthetic bone may be particularly desirable when younger individuals are involved. Remodeling can be achieved when defects are filled with osteoinductive (bone-forming) materials.

The formation of hydroxyapatite with the particulate agglomerate composition of the present invention *in vivo* is important in a variety of circumstances like gap filling and bone fragment st abilization. A synthetic material to fill bone defects that is compatible with decalcified bone has not been before identified and there has been a long felt need in the art. The initiation of any bone remodeling procedure will occur more rapidly if a hydroxyapatite-based prosthesis can be placed into close or microscopic apposition with bone. As a result of the present invention, the present method is amenable to placement of a bone-like composition of the reactants into close apposition with bone. This would eliminate the existence of a macroscopic gap between a synthetic hydroxyapatite-based prosthesis and bone or to fill gaps between a metallic prosthesis and bone. The development of union would be promoted in both instances, as a result of the present invention.

## SUMMARY OF THE INVENTION

The present invention is directed to a dry particulate agglomerate composition capable of producing phase-pure hydroxyapatite without additional sources of calcium and/or phosphate in the presence of an aqueous liquid consisting essentially of agglomerates, each individual agglomerate being a homogenous mixture of (i) $CaHPO_4 \cdot 2H_2O$ particles and (ii) particles of a calcium source comprising $Ca_4(PO_4)_2O$ wherein said $CaHPO_4 \cdot 2H_2O$ and said calcium source comprising $Ca_4(PO_4)_2O$ are present in an amount sufficient to form phase-pure HAp having a Ca/P ratio of 1.5 to 1.67 within 1 hour. The dry particulate agglomerate composition can be used in preparing a bone-like substitute composition or for a dental composition for filling teeth. The dry particulate agglomerate composition forms phase pure

hydroxyapatite upon setting in a clinically relevant time *in vivo* while being biocompatible and possessing mechanical properties in the range of those exhibited by natural bone and tooth materials. The synthetic bone-like composition is also conducive to the rapid ingrowth of bone.

The present invention is also directed to an aqueous composition which comprises

i.) as a solids component

(A) a dry particulate agglomerate composition which consists essentially of agglomerates, each of said agglomerates being a homogenous mixture of $CaHPO_4 \cdot 2H_2O$ particles and particles of a calcium source comprising $Ca_4(PO_4)_2O$, said agglomerate being prepared by mixing (a) $CaHPO_4 \cdot 2H_2O$ particles with (b) particles of a calcium source comprising $Ca_4(PO_4)_2O$ in (c) a non-aqueous liquid, and removing said non-aqueous liquid; said $CaHPO_4 \cdot 2H_2O$ and said calcium source comprising $Ca_4(PO_4)_2O$ are present in an amount sufficient to form phase-pure hydroxyapatite having a Ca/P ratio of from 1.5 to 1.67; and

(B) a polymeric material which is capable of promoting mineralization of hydroxyapatite from said particles (A) and wherein the weight ratio of (A)/(B) is from 100/0 to 40/60, respectively: and

(ii.) a physiologically acceptable aqueous liquid;

said aqueous composition having a liquid-to-total solids weight ratio of 0.15 to 1.5:

and wherein said aqueous composition reacts to form phase pure hydroxyapatite from component (A) at physiological temperatures within 1 hour, and a method of making said composition.

Another object of the present invention is to provide a physiologically useful hydroxyapatite composition comprising hydroxyapatite prepared from said dry particulate agglomerate composition as described above, and optionally a polymeric material capable of

promoting mineralization of hydroxyapatite, for repairing teeth or for bone grafting and implantation, wherein said composition meets the requirements of a bone-like material and which cure to form phase-pure hydroxyapatite in a surgically relevant time, preferably under 1 hour.

A further object of the present invention is to provide a method of filling in any bone defects with, or implanting a biocompatible and structurally strong, easily formed synthetic bone-like composition by using the dry particulate agglomerates of the present invention.

Still a further object of the present invention is to provide a method of making a particulate agglomerate composition capable of producing phase-pure hydroxyapatite without additional sources of calcium and/or phosphate in the presence of an aqueous liquid, and which, optionally, can be premixed with a polymeric material capable of promoting mineralization of hydroxyapatite prior to use in a body which comprises:

(a) mixing (i) $CaHPO_4 \cdot 2H_2O$ particles with (ii) particles of a calcium source comprising $Ca_4(PO_4)_2O$, in a non-aqueous liquid wherein said $CaHPO_4 \cdot 2H_2O$ and said calcium source comprising $Ca_4(PO_4)_2O$ are present in an amount sufficient to form phase-pure hydroxyapatite having a Ca/P ratio of 1.5 to 1.67, to form said particulate agglomerates; and

(b) collecting said particulate agglomerates.

Still a further object of the invention is to provide a kit comprising a container comprising a dry particulate agglomerate composition capable of producing phase-pure hydroxyapatite without additional sources of calcium and/or phosphate in the presence of an aqueous liquid consisting essentially of agglomerates, each individual agglomerate being a homogenous mixture of (i) $CaHPO_4 \cdot 2H_2O$ particles and (ii) particles of a calcium

source comprising $Ca_4(PO_4)_2O$ wherein said $CaHPO_4 \cdot 2H_2O$ and said calcium source comprising $Ca_4(PO_4)_2O$ are present in an amount sufficient to form phase-pure HAp having a Ca/P ratio of 1.5 to 1.67, which can be stored over long periods of time; and which can be utilized for the preparation of a hydroxyapatite composition for a dental or surgical procedure which will benefit from using said hydroxyapatite composition.

This and other objects and advantages of this invention are described and are particularly delineated in the appended claims.

## BRIEF DESCRIPTION OF THE FIGURE

FIG. 1 is a phase diagram showing stability regions for calcium phosphates.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

One embodiment of the present invention is directed to a method of producing a particulate agglomerate composition, each of said particulate agglomerates being a homogenous mixture of (i) $CaHPO_4 \cdot 2H_2O$ particles and (ii) particles of a calcium source comprising $Ca_4(PO_4)_2O$, which are capable of forming hydroxyapatite in the absence of additional sources of calcium and/or phosphate, which comprises the steps of:

(a) mixing (i) $CaHPO_4 \cdot 2H_2O$ particles with (ii) particles of a calcium source comprising $Ca_4(PO_4)_2O$ in a non-aqueous liquid, wherein said $CaHPO_4 \cdot 2H_2O$ and said calcium source comprising $Ca_4(PO_4)_2O$ are present in an amount sufficient to form phase pure hydroxyapatite having an overall Ca/P ratio of 1.5 to 1.67; and

(b) collecting said particulate agglomerates.

By homogenous mixture is meant complete homogeneity of the $CaHPO_4 \cdot 2H_2O$ particles and the particles of the

calcium source comprising $Ca_4(PO_4)_2O$ in each individual particle capable of producing phase pure hydroxyapatite having a Ca/P ratio of 1.5 to 1.67. That is, the particles of $CaHPO_4 \cdot 2H_2O$ and the particles of the calcium source comprising $Ca_4(PO_4)_2O$ are completely dispersed among each other within each individual agglomerate.

By particles of a calcium source comprising $Ca_4(PO_4)_2O$ is meant particles consisting of $Ca_4(PO_4)_2O$ as the sole calcium source, or particles consisting essentially of $Ca_4(PO_4)_2O$ and CaO, or particles consisting essentially of $Ca_4(PO_4)_2O$ and $Ca_3(PO_4)_2$.

The mol ratio of $CaHPO_4 \cdot 2H_2O$ to $Ca_4(PO_4)_2O$ sufficient to form phase-pure HAp having a Ca/P ratio of 1.5 to 1.67 is 2/1 to 1/1, respectively, when the sole calcium source is $Ca_4(PO_4)_2O$.

When CaO or $Ca_3(PO_4)_2$ is present with the $Ca_4(PO_4)_2O$ as the calcium source, the upper limit in the mole ratio should be 0.2 (CaO or $Ca_3(PO_4)_2$ / $Ca_4(PO_4)_2O$).

For example, when the calcium source consists of CaO and $Ca_4(PO_4)_2O$ in a mole ration of 0.2, then the mole proportion of $CaHPO_4 \cdot 2H_2O$ to CaO to $Ca_4(PO_4)_2O$ is 2.5:0.2:1, respectively, to produce HAp having a Ca/P of 1.5. When the calcium source consists of CaO and $Ca_4(PO_4)_2O$ in a mole ration of 0.2, then the mole proportion of $CaHPO_4 \cdot 2H_2O$ to CaO to $Ca_4(PO_4)_2O$ is 1.38:0.2:1, respectively, to produce HAp having a Ca/P of 1.67.

For example, when the calcium source consists of $Ca_3(PO_4)_2$ and $Ca_4(PO_4)_2O$ in a mole ratio of 0.2, the mole proportion of $CaHPO_4 \cdot 2H_2O$ to $Ca_3(PO_4)_2$ to $Ca_4(PO_4)_2O$ is 2:0.2:1, respectively, to produce HAp having a Ca/P of 1.5. When the calcium source consists of $Ca_3(PO_4)_2$ and $Ca_4(PO_4)_2O$ in a mole ratio of 0.2, the mole proportion of $CaHPO_4 \cdot 2H_2O$ to $Ca_3(PO_4)_2$ to $Ca_4(PO_4)_2O$ is 0.88:0.2:1, respectively, to produce a HAp having a Ca/P ratio of 1.67.

While not being bound to any theories, important advantages of preparing the inventive particulate agglomerate composition, wherein each particulate agglomerate is a homogenous mixture of $CaHPO_4 \cdot 2H_2O$ particles and particles of a calcium source comprising $Ca_4(PO_4)_2O$, which are capable of forming phase pure hydroxyapatite, of the present invention in a non-aqueous liquid are the following. The presence of a non-aqueous liquid enhances the mixing by affecting the surface charges on the particles allowing a much higher degree of mixing than can be achieved by dry blending, dry mixing or dry grinding of the mixture.

After the inventive particulate agglomerate composition is achieved, the non-aqueous liquid is removed from the particles by, e.g., filtration. The process of the final liquid removal is by evaporation.

By way of explanation of the prior art, all acidic calcium phosphates and $Ca_4(PO_4)_2O$ dissolve incongruently. This means that when a crystal of $CaHPO_4$ or $Ca(H_2PO_4)_2.H_2O$ dissolves, it creates a solution which is supersaturated in another calcium phosphate before the solution becomes saturated with respect to the dissolving crystal (see Figure 1). This means that the other calcium phosphate precipitates on the surface of the dissolving crystal. For $CaHPO_4$, $CaHPO_4.2H_2O$ and for $Ca_4(PO_4)_2O$, the other calcium phosphate is hydroxyapatite. For $Ca(H_2PO_4)_2.H_2O$, the other calcium phosphate is $CaHPO_4.2H_2O$. Therefore, when these calcium phosphates are used as reactants for hydroxyapatite, their dissolution causes hydroxyapatite to form on their surfaces, reduces the effective surface area for dissolution, and slows down reaction.

However, when $CaHPO_4 \cdot 2H_2O$ particles and particles of a calcium source comprising $Ca_4(PO_4)_2O$ are mixed with the intimacy achieved by using a non-aqueous liquid to form particulate agglomerate composition as in the present invention, this effect is minimized. Such an inventive

particulate agglomerate composition cannot be formed by dry intergrinding or dry mixing as in the prior art. The inventive particulate agglomerate composition must be formed by mixing in the presence of a non-aqueous liquid. Since the produced particulate agglomerates do not contain protons in the form of $H_2PO_4$, which could initiate further reactivity, the dry particulate agglomerate composition can be stored for an unlimited period of time, if kept dry. The extended shelf-life of such a product contributes to its advantage as a dental or bone-like substitute which can be constituted in an operating theater in a surgically relevant timeframe.

The time of mixing in the non-aqueous liquid is not critical so long as the time is sufficient to provide agglomerates, wherein each agglomerate is a homogeneous mixture of the $CaHPO_4 \cdot 2H_2O$ particles and particles of a calcium source comprising $Ca_4(PO_4)_2O$, and wherein the individual agglomerates can form phase-pure hydroxyapatite within 1 hour, and particularly within 30 minutes in the presence of an aqueous liquid.

The dry particulate agglomerate composition of the present invention can be further mixed with a polymeric material which acts as a substrate for hydroxyapatite mineralization and can be further sterilized by heat, by gamma radiation or by ethylene oxide.

Formation of hydroxyapatite *in vivo* from the particulate agglomerate composition of the present invention allows the reactants to be placed in a very close association with the bone or tooth being repaired. This cannot be achieved by using a preformed hydroxyapatite prosthesis.

Hydroxyapatite Formation - As an example of using the dry particulate agglomerate composition of the present invention, calcium deficient hydroxyapatite (having a Ca/P ratio of 1.5) can be formed from the intimate mixture of particles by the following reaction:

EQ [1]

$$6CaHPO_4 \cdot 2H_2O + 3Ca_4(PO_4)_2O \xrightarrow{H_2O} 3Ca_9HPO_4(PO_4)_5OH + 13H_2O$$

To prepare the inventive particulate agglomerate composition, the mixing must take place in a non-aqueous liquid. Preferably the non-aqueous liquid is of sufficient volatility so as to be easily removed by evaporation after the formation of the intimate mixture of particles. The non-aqueous liquid is not a solvent for said $CaHPO_4 \cdot 2H_2O$ or $Ca_4(PO_4)_2O$. Moreover, the non-aqueous liquid does not react directly with the $CaHPO_4 \cdot 2H_2O$ particles and particles of a calcium source comprising $Ca_4(PO_4)_2O$. When it is desired to produce only hydroxyapatite complete at reaction, the mole ratio amount of said $CaHPO_4 \cdot 2H_2O$ and said calcium source comprising $Ca_4(PO_4)_2O$ are selected to achieve Ca/P ratios from 1.5 to 1.67.

The amount of non-aqueous liquid used in preparing the inventive individual particles of the present invention is not critical. An amount of 1 volume of reactants to 2 volumes of non-aqueous liquid may be used as a guideline. Moreover, the non-aqueous liquid does not have to be non-toxic since the non-aqueous liquid is completely removed from the inventive individual particles prior to use *in vivo*.

By non-aqueous liquid is meant, any liquid which will not dissolve the $CaHPO_4 \cdot 2H_2O$ particles or the particles of the calcium source comprising $Ca_4(PO_4)_2O$, will not react with either type of the particles, and will not allow the particles to react with each other.

Examples of such non-aqueous liquids include, but are not limited to, liquid aliphatic hydrocarbons which may be straight, branched or cyclic, and aromatic hydrocarbons which may be further substituted with functional groups such as halogens. Examples of

liquid aliphatic hydrocarbons, include, but are not limited to substituted or unsubstituted liquid alkanes, and liquid alkenes. Examples include but are not limited to butane, pentane, hexane, heptane, $CCl_4$, $CH_2Cl_2$ and $C_2Cl_6$.

The temperature range for reacting $Ca_4(PO_4)_2O$ with $CaHPO_4 \cdot 2H_2O$ is not critical, so long as the temperature is below the decomposition temperature of the $CaHPO_4 \cdot 2H_2O$.

The particle size of the $CaHPO_4 \cdot 2H_2O$ particles and $Ca_4(PO_4)_2O$ particles are in the range of from 0.05 to 10 μm, and preferably, 0.05 to 5 μm.

The average particulate agglomerate size is in the range of 1 μm to 15 μm.

Figure 1 shows the compositional relationships between $CaHPO_4$ and HAp to be peritectic reactions. As a result, the hydrolysis of $CaHPO_4$ results in it being overgrown by hydroxyapatite or intermediate products and causing the complete reaction to hydroxyapatite to become undesirably diffusionally controlled. For example, the hydrolysis of $CaHPO_4$ occurs as follows:

$$9CaHPO_4 \xrightarrow{H_2O} Ca_9(HPO_4)(PO_4)_5OH + 6H^+ + 3HPO_4^{2-} \qquad \text{EQ [2]}$$

even in the presence of $Ca_4(PO_4)_2O$. The hydroxyapatite formed does so as a coating on the $CaHPO_4$ reactant and results in a lengthy period of time to complete reaction. A similar set of events occur when one of the initial reactants is $CaHPO_4 \cdot 2H_2O$ except when incorporated into the particulate agglomerate composition of the present invention. $CaHPO_4$ cannot be used in the inventive particulate agglomerate composition as shown in the Comparative Experiment which follows.

According to the present invention mixing of $CaHPO_4 \cdot 2H_2O$ with a calcium source comprising $Ca_4(PO_4)_2O$ in a non-aqueous liquid results in the formation of particulate agglomerates, each agglomerate of which is a homogenous mixture of $Ca_4(PO_4)_2O$ and a calcium source comprising $CaHPO_4 \cdot 2H_2O$. When the particulate agglomerate composition of the present invention is mixed with a physiologically acceptable aqueous liquid such as sterile water for injection, or normal saline for injection, these combinations of reactants react completely to form hydroxyapatite within minutes to an hour without the formation of intermediates. Preparing the inventive particulate agglomerate composition with a non-aqueous liquid is required to obtain the degree of intimacy needed to avoid the extended periods of reaction caused by such overgrowth of HAp.

The type of reaction vessel or its size for preparing the particulate agglomerate composition of the present invention is not critical, and can be any vessel capable of holding the $CaHPO_4 \cdot 2H_2O$, $Ca_4(PO_4)_2O$ and non-aqueous liquid so as to allow the mixing to take place. An example of such a vessel is any commercially available polyethylene bottle. Examples of pellets to facilitate mixing of the individual particles include, but are not limited to, zirconia, alumina and polycarbonate pellets.

The present invention was developed because mechanical mixtures of dry calcium sources and acidic phosphate source e.g., $CaHPO_4$ and $Ca_4(PO_4)_2O$, or $Ca_4(PO_4)_2O$ and a calcium source comprising $CaHPO_4 \cdot 2H_2O$ in suitable proportions, to form hydroxyapatite as shown in U.S. Patent 4,612,053 to W. Brown and Chow requires an extended period of time to reach completion while those of $Ca(H_2PO_4)_2 \cdot H_2O$, $H_3PO_4$ or $H_3PO_4 \cdot 1/2H_2O$ with $Ca_4(PO_4)_2O$ form intermediates which may also persist for lengthy

periods, which is undesirable in the average time for a dental or surgical procedure.

When hydroxyapatite is produced according to the present invention and is used to repair dentitia, the inventive particulate agglomerate composition can be used alone, or additional components can be mixed therewith to alter the physical properties of the produced hydroxyapatite.

When the particulate agglomerate composition of the present invention is used to produce a synthetic bone-like HAp composition, polymeric materials capable of allowing mineralization of hydroxyapatite can be mixed therewith in an amount sufficient to achieve any of a number of desired effects. Such desired effects can be, e.g., bone formation, enhanced resorption, growth of osteoblasts and/or osteoclasts, angiogenesis, cell entry and proliferation, mineralization, altering mechanical properties, or the like.

In defining the aqueous composition embodiment of the invention, by solids component is meant the total weight of (A) inventive particulate agglomerate composition; or the total weight of (A) plus the polymeric material capable of promoting mineralization of hydroxyapatite (B).

The weight ratio of (A) to (B) is in the range of 100/0 to 40/60 respectively, particularly 99.99/0.01 to 60/40 respectively, and more particularly 99/1 to 60/40 respectively, depending on the polymeric material employed. When the polymeric material is bone associated protein, and particularly treated insoluble collagen, the weight ratio of (A)/(B) is 99/1 to 60/40.

By liquid-to-total solids weight ratio is meant the ratio of the total weight of the physiologically acceptable aqueous liquid to the total weight of the solids present in the composition. The total weight of the solids will include the solids component described

in the preceding paragraph as well as any additional additives, such as fillers, bisphosponates, etc.

The physiologically acceptable aqueous liquid which is used to constitute formation of phase pure hydroxyapatite *in vivo* will be substantially pure, e.g., sterile water for injection, normal saline for injection or equivalents thereof.

Examples of such polymeric materials (B) can be synthetic polymers, or proteins known to be associated with bone, cartilage, or dentin.

With respect to synthetic polymeric materials those which contain functional groups capable of being hydrolyzed by $H_2O$, such as carboxyl, amine or sulfonate groups, are useful so long as the functional groups on the polymer are capable of forming salt bridges with calcium and phosphate. Examples of water soluble synthetic polymers, include but are not limited to polyacrylic acid and polyacrylic-itaconic acid co-polymers.

Examples of such polymeric bone proteins include collagen, particularly Type 1, osteonectin ZHS-glycoproteins, sialoproteins (BSP), bone-Gla-protein (BGP) matrix-Gla-protein bone proteoglycan bone phosphoglycoprotein, bone phospho-protein, skeletal growth factor, and the like.

Examples of such polymeric cartilage proteins include chondrocalcining proteins.

Examples of such polymeric dentin or enamel proteins include glycoproteins such as Gla protein, phosphophoryn, amelogelnin, and enamelin.

A particularly preferred polymeric material is insoluble collagen which has been treated and which is prepared as follows.

Collagen Treatment Process - Collagen treatment serves three functions in contributing to the development of hydroxyapatite-collagen bone replacement composites. It allows coarse collagen granules to be

converted into fibrils. It orients these fibrils in a three dimensionally interconnected network. This network structure provides a template on which hydroxyapatite formation occurs. Thus, the treated collagen prepared by the process of the present invention also serves to facilitate hydroxyapatite formation *in vivo*.

The collagen treatment method is achieved by a combination of physical, chemical and mechanical actions. Granular insoluble collagen obtained from, e.g., Sigma Chemical Company, is suspended in an acidic solution and the collagen subjected to a mechanical action, such as from a commercial blender (step a). The organic acid breaks the hydrogen bonds between fibrils and allows their expansion in solution by hydration. The acid may also be selected to control the rate of hydroxyapatite formation (discussed below). Mechanical shear promotes the physical separation and expansion of the fibrils. After this processing step, the suspension is rapidly frozen in liquid nitrogen (step b). Freezing in other low boiling point liquids (He, $H_2$, $O_2$, Ar, etc., or solids ($CO_2$) will also work. Expansion of water present on freezing this suspension assists in the expansion and separation of the dense tendinous collagen structure. The frozen mass is then thawed and mechanically sheared for a second time to further the separation of the fibrils (step c). This process can be repeated as many times as is necessary.

After reblending, the suspension is again frozen using liquid nitrogen and then freeze-dried. Freeze drying allows the removal of both the water and the organic acid. This process results in a three dimensional organic matrix consisting of oriented submicron fibrils interconnected with fibrils and collagenous membranes. This matrix is a result of the freezing direction, namely the fibrils align in the direction of the solidification front. The extended

structure provides a network for the formation of hydroxyapatite. This structure has pores and channels ideal for the incorporation of the hydroxyapatite. In addition, the pore and channel dimensions can be easily controlled by altering the acid concentration and water content in step (a). Thus, altering the acid concentration and water content to control the collagen microstructure can also control the resulting properties of the synthetic bone. The treated collagen has pore diameters in the range of from 2 μm to 100 μm.

A number of features are associated with treating insoluble collagen. First, the insoluble collagen can be processed quite rapidly. Insoluble collagen harvested by known surgical methods from another part of the individual to be treated can be used as a result. Collagen obtained from another individual can also be treated.

Other materials may be added to the aqueous composition of the present invention to provide for specific types of mechanical physical properties. Both inorganic and organic fibrous materials can be employed. Examples include hydroxyapatite fibers and biodegradable organic fibers such as polyglycolic acid.

Fillers can be mixed with the inventive particulate agglomerate composition and, optionally, polymeric materials prior to reaction with physiologically acceptable aqueous liquid, in order to control the porosity, and increase the early strength of the compositions during mixing and surgery. Examples of fillers include preexisting HAp, $CaHPO_4$, $CaHPO_4$ coated with fluorapatite, $Ca_4(PO_4)_2O$ coated with $CaHP0_4$. The filler can be formulated to any particle size, preferably between 15 to 75 μm. The proportion of filler, when present, can be as high as 65 wt% based on the weight of the solids component (A) and (B) of the composition.

Further, additional additives can be added which include, but are not limited to fluoride sources, antibiotics, surfactants, and dispersants in order to exert their intended effect.

With respect to the addition of antibiotic, the choice of antibiotic incorporated into the composition depends on the microorganisms to be treated, and is well within the level of skill in the art.

Effective amounts of antibiotics can be incorporated into the compositions in order to prevent or treat infections of the bone or dentitia or infections of surrounding tissue. Effective amounts are well known or can be readily ascertained by one skilled in the art.

Examples of antibiotics include, but are not limited to penicillin, cephalothin, tobramycin, gentamycin, nafcillin, rifampin, clindamycin, polymyxin, metronidazole, chloramphenicol well known in the art as evidenced by the Physicians Desk Reference, 48th Edition, 1994.

Effective amounts of the antibiotics can be dissolved in the physiologically acceptable liquid or can be mixed with the inventive particulate agglomerate composition and, optionally, the polymeric material. The rate of release of antibiotics can be controlled by controlling the initial concentration of the antibiotic.

A hydroxyapatite tooth or bone substitute composition prepared by the present invention can be prepared by a number of methods.

In a first method, the polymeric material capable of promoting mineralization of hydroxyapatite can be added directly into the non-aqueous liquid used in forming the inventive particulate agglomerate composition prior to removal of the non-aqueous liquid.

In a second method, the inventive particulate agglomerate composition may be mixed with a polymeric material capable of promoting mineralization of

hydroxyapatite in an aqueous phase, prior to use *in vivo*.

When preparing the composition of the present invention, the liquid-to-total solids weight ratio is from 0.15 to 1.5 (liquid-to-total solids component), preferably 0.25 to 1.0.

The additives can be added at any time during the process of preparing the aqueous composition, up until use of the composition *in vivo* in the relevant procedure.

Regardless of which method is used, the resultant composition can be utilized in mass as a monolith or can be manipulated with a polymeric material, such as collagen, to achieve a net anisotropic orientation.

In preparing the particulate agglomerate composition of the present invention for use *in vivo*, if the first method is used, the non-aqueous liquid is removed by evaporating at ambient temperature. If the second method is used, a blend of the inventive particulate agglomerate composition and polymeric material in aqueous phase is frozen in liquid nitrogen and then freeze dried. After the non-aqueous liquid or the frozen water is removed, the resulting blend is crushed lightly to yield a slightly fibrous powder. This powder can then be sterilized and stored dry ready for constitution during the relevant procedure. When needed, it can be hand or mechanically mixed with 150% of its weight of a physiologically acceptable liquid, such as sterile water for injection, or normal saline. This mixing step occurs prior to anticipation of use *in vivo*.

Compositional Adjustments - Sodium and carbonate can be incorporated into the compositions by mixing $CaHPO_4 \cdot 2H_2O$ and a calcium source comprising $Ca_4(PO_4)_2O$ described above in the presence of up to 7% by weight of particulate $NaHCO_3$, based on the weight of the particles

of an intimate mixture of $CaHPO_4 \cdot 2H_2O$ and a calcium source comprising $Ca_4(PO_4)_2O$ to obtain a composition which is as close as possible to natural bone and/or teeth. The incorporation of carbonate and sodium into the hydroxyapatite composition demonstrates that the $Na^+$ and $CO^2_3$- concentrations in solution decreased linearly as the formation of hydroxyapatite proceeds, attaining concentrations of a few millimoles at complete reaction. Use of 7% by weight of $NaHCO_3$ based on the weight of the particles of HAp resulted in a hydroxyapatite composition containing approximately 3.6 wt% $CO_3$. As a result of incorporating up to 7% by weight of Na and carbonate and particularly, 1 to 7% by weight of Na and carbonate, a hydroxyapatite composition close to that of native bone can be formed by a reaction which is physiologically compatible *in vivo*. Therefore, a mineral phase close in composition to that of natural bone and teeth can be formed *in vivo*. Developing prostheses which will remodel *in vivo* in a manner similar to that of natural bone and teeth.

A variety of hydroxyapatite compositions may be achieved, depending on desired application of the dentist or surgeon. Alkali phosphates can be added to the aqueous solution to influence the kinetics of reactant dissolution, for example by making such dissolution increasingly congruent. A fluoride source, for example, NaF or $CaF_2$, can be added to form fluoride substituted apatite in which can be present to partially or completely substitute for OH. Moreover, various acids, e.g., acetic acid and citric acid can be added to the aqueous phase, which do not enter the apatite structure, but which influence the rate of hydroxyapatite formation. Specifically, small additions, e.g., (from 15 to 150mM) of acetic acid to the aqueous phase accelerate the onset of hydroxyapatite formation while small additions of citric acid (from 15 to 150 mM) delay the onset of hydroxyapatite formation.

Such additives provide the means to control the rate of hydroxyapatite formation *in vivo*. The presence of such additives may be synergistically associated with the presence of the processed collagen (as discussed).

Having generally described the invention, the following specific examples are included for the purpose of illustration only and not intended to limit the scope of the invention. For ease of calculation, the liquid components in the following examples are rounded off to have a density of 1.0 g/ml. All liquid-to-total solids ratios are by weight.

**EXAMPLE 1**

**Preparation of a Particulate Agglomerate Composition Capable of Forming HAp having a Ca/P Ratio of 1.5 (calcium deficient HAp)**

In a typical run to prepare a particulate agglomerate composition which will be employed to form $Ca_9HPO_4(PO_4)_5OH$ (HAp having a Ca/P ratio of 1.5) *in vivo*, 172 g of $CaHPO_4 \cdot 2H_2O$ (Fisher Chemical Co.) having a particle size of from 0.5 μm to 5 μm is mixed with 183 g of $Ca_4(PO_4)_2O$ having a particle size of from 0.5 μm to 5 μm. The $Ca_4(PO_4)_2O$ is prepared by firing an intimate mixture of equimolar amounts of $CaCO_3$ and $CaHPO_4$ powders to 1400°C for 4 hours. After cooling the $Ca_4(PO_4)_2O$ is ground to a particle size of 0.5 μm to 5 μm using standard powder preparation techniques. This material is then mixed with $CaHPO_4 \cdot 2H_2O$ which has been ground to having a particle size of from 1 μm to 5 μm using standard powder preparation techniques, by placing it and $Ca_4(PO_4)_2O$ in a 1-liter polyethylene bottle with approximately 400 ml of heptane.

Approximately 250g of zirconia or alumina, or 100g polycarbonate pellets are added and the bottle is

sealed. The presence of the pellets facilitate agglomerate formation. The bottle is then placed in any shaking or rolling device.

There is a negligible reduction in particle size during reaction in heptane; therefore, this intimate mixing step does not involve grinding. The particulate agglomerate composition is formed according to the present process and cannot be duplicated by grinding in air, grinding in aqueous solvents, or grinding in water. After mixing, the slurry of particulate agglomerates and the pellets is placed on a filter funnel and the bulk of the heptane is removed by vacuum suction and can be reused after distillation. The pellets are then removed. Any material which adheres to pellets is removed by washing in heptane. Heptane not removed by vacuum suction is allowed to evaporate, either at room or slightly elevated temperature. Evaporation may also be carried out in vacuum.

When reacted with a physiologically acceptable aqueous liquid at physiological temperature, the particulate agglomerate composition of the invention achieves a substantial degree of completion to HAp in 15 minutes or less and converts completely to HAp in periods of time which range from less than 30 minutes to less than 1 hour.

## EXAMPLE 2

### Preparation of Mixed Calcium Sources

Calcium sources comprising of a mixture of $Ca_4(PO_4)_2O$ and CaO or of $Ca_4(PO_4)_2O$ and $Ca_3(PO_4)_2$ are prepared at high temperature by processing similar to that described in Example 1, above. This is accomplished by varying the proportions of calcium-containing and phosphate-containing reactants. Mixtures composed of $Ca_4(PO_4)_2O$ and CaO require Ca/P ratios greater than 2 while mixtures of $Ca_4(PO_4)_2O$ and $Ca_3(PO_4)_2$ require ratios less than 2. Mixtures of $Ca_4(PO_4)_2O$ and $Ca_{10}(PO_4)_6O$

(oxyapatite) are prepared when the reaction temperature is maintained below about 1275°C. When the high temperature reaction time is shorter than about 4 hours and when the reaction temperature is below about 1300°C, mixtures of $Ca_4(PO_4)_2O$, CaO, and $Ca_{10}(PO_4)_6O$ are prepared. Non-equilibrium mixtures of CaO and $Ca_3(PO_4)_2$ are produced if the reaction temperature is above about 1600°C, which is high enough to attain a liquid phase.

The inventive particulate agglomerate composition can also prepared from these calcium sources by mixing them with $CaHPO_4 \cdot 2H_2O$ particles in a similar fashion as set forth in Example 1. Of course, the proportions of the reactants must be adjusted to ensure that the particles formed have a Ca/P ratio in the range of 1.5 to 1.67 which can be readily ascertained by one skilled in the art.

## EXAMPLE 3

### Collagen processing

Insoluble collagen granules (Sigma) are used as the starting material. Approximately 10 g of insoluble collagen granules are placed in 100 ml of solution of acetic acid (50 mM). This mixture is allowed to sit for approximately 16 hours. The mixture is then mechanically sheared in a Waring commercial blender for 5 minutes. The mixture is then rapidly frozen in liquid nitrogen. Freezing assists the separation of the collagen fibers. The mixture is then allowed to thaw and refrozen in liquid nitrogen a second time. This process may be repeated as many times as is desired, but in the detailed example (to be described) it was done twice. After the last freezing cycle, the frozen mass is freeze dried. Freeze drying removes both water and acetic acid such that, if the collagen is immersed in water at a liquid-to-solid weight ratio of 1 after drying is complete, the pH does not drop below 5.5.

## EXAMPLE 4

### Composite formation

Collagen, treated as described above in Example 3 is mixed with the particulate agglomerate composition as described above in Example 1. Three proportions, expressed in terms of the hydroxyapatite:collagen volume ratios, of 2:1, 5:1 and 10:1 is prepared. A typical mix weighs 50 grams. The proportions of $CaHPO_4 \cdot 2H_2O$ and $Ca_4(PO_4)_2O$ used is selected to produce calcium deficient hydroxyapatite, $Ca_9HPO_4(PO_4)_5OH$, at complete reaction, see EQ [1] and Example I.

## EXAMPLE 5

### Production of HAp/Collagen Composition Containing Tobramycin Sulfate

100 gm of the particulate agglomerate composition prepared according to Example 1 is mixed with 4.7 gm of treated collagen prepared according to Example 3 (solid component). A stock saline solution of tobramycin sulfate is prepared by dissolving 40 mg tobramycin sulfate per ml of normal saline. 1.2 gm of the tobramycin sulfate-containing saline (liquid component) is added to 3.0 gm of the solid component to form a mixture (a liquid-to-total solids weight ratio of 0.4). The mixture is mixed for 2 minutes at room temperature and surgically implanted into a 15 mm cranial defect in a rabbit. The tobramycin sulfate-to HAp weight ratio is approximately 16 mg of tobramycin sulfate per gram of phase pure HAp.

## EXAMPLE 6

### Production of HAp Containing Tobramycin Sulfate

The particulate agglomerate composition is prepared according to Example 1 in order to produce calcium deficient HAp (Ca/P = 1.5) (solids component). No treated collagen is used in this example. 1.02 gm of

the tobramycin sulfate stock saline solution prepared according to Example 7A is mixed with 3.0 gms of the solid component (a liquid-to-total solids weight ratio of 0.34) for two minutes at room temperature. The composition is surgically implanted in 15 mm cranial defects in a rabbit.

A wide range of rates of antibiotic release (designed to maintain a release rate in the therapeutic range of a selected period of time) can be achieved.

## EXAMPLE 7

### Production of HAp/Collagen + HAp-Coated $CaHPO_4$ Filler to Control Porosity

Coating of $CaHPO_4$ filler particles with HAp is carried out as follows: 100 gm of $CaHPO_4$ particles having a particle size of 35 µm are placed in 400 ml of distilled water and stirred slowly. After about 16 hours, when the pH of the water reaches approximately 4.6, the produced HAp-coated $CaHPO_4$ filler particles are filtered from solution using filter paper and a Buchner funnel. These HAp-coated $CaHPO_4$ filler particles are then washed with approximately 100 ml of distilled water and dried in a desiccator over Drierite ($CaSO_4 \cdot 1/2H_2O$).

At the time of surgery, 0.9 gm of normal saline solution is mixed with 1.2 gm of HAp-coated $CaHPO_4$ filler particles and with 1.8 gm of a mixture of solid components containing the particulate agglomerate composition prepared according to Example 1 and treated collagen prepared according to Example 3 wherein said mixture is obtained by mixing 100 gm of the particulate agglomerate composition of Example 1 and 4.7 gm of treated collagen of Example 3. The liquid-to-total solids (solids component + filler) weight ratio is 0.30. Mixing of the liquid and the total solids is carried out for 2 minutes at room temperature. The resultant composition is surgically implanted in a 15 mm cranial defect in a rabbit.

## EXAMPLE 8

### Production of HAp/Collagen + FAp-Coated $CaHPO_4$ Filler to Control Porosity and Delivery Fluoride

Coating of $CaHPO_4$ filler particles with fluoroapatite (FAp) is carried out as follows: 200 gm of $CaHPO_4$ particles having a particle size of 35 µm are placed in 1000 ml of distilled water to which 0.44 gm of NaF has been added and stirred slowly for 16 hours at room temperature. The produced FAp-coated $CaHPO_4$ filler particles are filtered from solution using filter paper and a Buchner funnel. These FAp-coated $CaHPO_4$ filler particles are then washed with approximately 200 ml of distilled water and dried in a desiccator over Drierite ($CaSO_4 \cdot 1/2H_2O$).

At the time of surgery, 0.9 gm of normal saline solution is mixed with 1.2 gm of FAp-coated $CaHPO_4$ filler particles and with 1.8 gm of a mixture of solid components containing the particulate agglomerate composition according to Example 1 and treated collagen prepared according to Example 3 wherein said mixture is obtained by mixing 100 gm of the particulate agglomerate composition of Example 1 and 4.7 gm of treated collagen of Example 3. The liquid-to-total solids (solids component + filler) weight ratio is 0.30. Mixing of the liquid and the total solids is carried out for 2 minutes at room temperature. The resultant composition is surgically implanted in a 15 mm cranial defect in a rabbit.

## EXAMPLE 9

### HAp/Collagen + HAp-Coated $Ca_4(PO_4)_2O$ Filler to Control Porosity

Coating of 57 µm $Ca_4(PO_4)_2O$ particles (TetCP) with $CaHPO_4$ is carried out as follows: 57.6 gm of 85% $H_3PO_4$ solution are mixed with approximately 400 ml of distilled water and heated to 80°C. 412 gm of 57 µm

$Ca_4(PO_4)_2O$ particles are rapidly added to the $H_3PO_4$ solution while stirring. The solution is stirred for 4 hours at 80°C after which the particles are filtered from this 80°C solution using filter paper and a Buchner funnel. These $CaHPO_4$-coated $Ca_4(PO_4)_2O$ filler particles are then dried in a desiccator over Drierite ($CaSO_4 \cdot 1/2H_2O$).

At the time of surgery, 0.9 gm of normal saline solution is mixed with 1.2 gm of $CaHPO_4$-coated TetCP filler particles and with 1.8 gm of a mixture of solid components containing the particulate agglomerate composition prepared according to Example 1 and treated collagen prepared according to Example 3 wherein said mixture is obtained by mixing 100 gm of the particulate agglomerate composition of Example 1 and 4.7 gm of treated collagen of Example 3. The liquid-to-total solids (solids component + filler) weight ratio is 0.30. The liquid used is normal saline. Mixing of the liquid and the total solids is carried out for 2 minutes at room temperature. The resultant composition is surgically implanted in a 15 mm cranial defect in a rabbit.

In contrast to the $CaHPO_4$ filler (Ca/P = 1) of Example 8 which is acidic, the composite TetCP/$CaHPO_4$ filler Ca/P = 1.8) used in this experiment is basic. The Ca/P ratio of 1.8 is selected because this ratio approximates the ratio in bone. This ratio is also selected because HAp formation at this ratio will theoretically take up carbonate. This composite filler has the theoretical capacity to form HAp by reaction in physiological fluids without added water. As a consequence, the implant should contain a lower proportion of porosity when the filler present has reacted to form HAp.

**EXAMPLE 10**

**HAp/Collagen + HAp-Coated $Ca_4(PO_4)_2O$ Filler to Control Porosity**

The above experiment in Example 9 is repeated except the proportions of constituents are changed to: 0.9 gm of normal saline solution is mixed with 1.8 gm of $CaHPO_4$-coated TetCP filler particles and with 1.2 gm of a mixture of solid components containing the particulate agglomerate composition prepared according to Example 1 and treated collagen prepared according to Example 3 wherein said mixture is obtained by mixing 100 gm of the particulate of Example 1 and 4.7 gm of treated collagen of Example 3. The liquid-to-total solids (solids component + filler) weight ratio is 0.30. The liquid used is normal saline. Mixing of the liquid and the total solids was carried out for 2 minutes at room temperature. The resultant composition is surgically implanted in a 15 mm cranial defect in a rabbit.

**EXAMPLE 11**

**Preparation of a Particulate Agglomerate Composition Capable of Forming HAp having a Ca/P Ratio of 1.67 (stoichiometric HAp)**

In a typical run to prepare a particulate agglomerate composition which will be employed to form $Ca_{10}(PO_4)_6(OH)_2$ (stoichiometric HAp) *in vivo*, 172 g of $CaHPO_4 \cdot 2H_2O$ is mixed with 366 g of $Ca_4(PO_4)_2O$. The $Ca_4(PO_4)_2O$ is prepared by firing an intimate mixture of equimolar amounts of $CaCO_3$ and $CaHPO_4$ powders to 1400°C for 4 hours. After cooling the $Ca_4(PO_4)_2O$ is ground to an average particle size of 0.5 μm to 5 μm using standard powder preparation techniques. This material is then mixed with $CaHPO_4 \cdot 2H_2O$ which has been ground to having an average particle size of from 1 μm to 5 μm using standard powder preparation techniques, by placing it

and $Ca_4(PO_4)_2O$ in a 1-liter polyethylene bottle with approximately 400 ml of heptane.

Approximately 325g of zirconia or alumina, or 130g polycarbonate pellets are added and the bottle is sealed. The presence of the pellets facilitates mixing. This bottle is then placed in any shaking or rolling device. The produced intimate mixture of $CaHPO_4 \cdot 2H_2O$ and a calcium source comprising $Ca_4(PO_4)_2O$ particles can then react rapidly in the presence of water to form hydroxyapatite *in vivo*.

After mixing, the slurry of particulate agglomerates and the pellets is placed on a filter funnel and the bulk of the heptane is removed by vacuum suction which can be reused after distillation. The pellets are then removed by hand picking. Any material which adheres to pellets is removed by washing in heptane. Heptane not removed by vacuum suction is allowed to evaporate, either at room or slightly elevated temperature. Evaporation may also be carried out in vacuum.

3 grams of the obtained particulate agglomerate composition is mixed with 1.2 gm of normal saline solution for 2 minutes at room temperature. The liquid-to-total solids weight ratio is 0.4. The resultant composition is surgically implanted in a 15 mm cranial defect in a rabbit.

**EXAMPLE 11A**

The above experiment in Example 11 is repeated except that the liquid-to-total solids weight ratio was reduced to 0.36. The mixture is implanted in a 15 mm cranial defect in a rabbit.

**EXAMPLE 12**

In this experiment, a 1.8 gm of the particulate agglomerate composition prepared according to Example 11 is mixed with 1.2 gm of FAp-coated $CaHPO_4$ filler prepared

according to Example 8, and 0.75 gm normal saline (a liquid-to-total solids weight ratio of 0.25). The mixture is implanted in a 15 mm cranial defect in a rabbit.

## EXAMPLE 13

### HAp + HEDP

Bisphosphonates are of interest as therapeutic agents to control the rate of bone resorption and the rate of tooth decay. A bisphosphonate, HEDP (1-hydroxyethylidene-1, 1-diphosphonic acid) $C_2H_8P_2O_7$, saline stock solution was prepared by mixing 2.5 gm of HEDP with 1000 ml of normal saline solution. 1.2 gm of the HEDP-containing saline solution is mixed with 3 grams of the particulate agglomerate composition prepared according to Example 1. The liquid-to-total solids weight ratio is 0.4. The resultant composition is surgically implanted in 15 mm cranial defects in a rabbit.

The HEDP-to-HAp weight ratio can be adjusted in three ways: (1) a larger amount of HEDP can be added to the saline solution, (2) the liquid-to-solids ratio can be increased, (3) the HEDP powder can be intermixed with the intimate mixture of particles prior to the addition of the liquid.

## COMPARATIVE EXPERIMENT

The rates of reaction for mixing $CaHPO_4 \cdot 2H_2O$ or $CaHPO_4$ were determined when mixed to form calcium deficient HAp and stoichiometric HAp according to the following reactions:

Exs. 1A & 1B. $2CaHPO_4 2H_2O + 2Ca_4(PO_4)_2O \Rightarrow Ca_{10}(PO_4)_6(OH)_2 + 4H_2O$

(calcium deficient HAp, Ca/P = 1.5)

Exs. 2A & 2B. $6CaHPO_4 + 3Ca_4(PO_4)_2O \Rightarrow 2Ca_9(HPO_4)(PO_4)_5OH + H_2O$

(calcium deficient HAp, Ca/P = 1.5)

Exs. 3A & 3B. $6CaHPO_4 2H_2O + 3Ca_4(PO_4)_2O \Rightarrow$

$2Ca_9(HPO_4)(PO_4)_5OH + 12H_2O$

(stoichiometric HAp, Ca/P = 1.67)

Exs. 4A & 4B. $2CaHPO_4 + 2Ca_4(PO_4)_2O \Rightarrow$

$Ca_{10}(PO_4)_6(OH)_2$

(stoichiometric HAp, Ca/P = 1.67)

The above reactants were mixed in two ways: (1) (Exs. 1A, 2A, 3A and 4A) dry intergrinding of the powdered $CaHPO_4 \cdot 2H_2O$ and $Ca_4(PO_4)_2O$ or $CaHPO_4$; and (2) (Exs. 1B, 2B, 3B and 4B) mixing $CaHPO_4 \cdot 2H_2O$ with $Ca_4(PO_4)_2O$ or $CaHPO_4$ in the non-aqueous liquid heptane, followed by removing the non-aqueous liquid by filtration and its evaporation. Thus, a total of 8 experiments were carried out to determine the rate of HAp formation when these combinations of reactants were mixed with water at 37.4°C: formation of stoichiometric and calcium deficient HAp using mixtures of $CaHPO_4 \cdot 2H_2O$ or $CaHPO_4$ with $Ca_4(PO_4)_2O$ when the mixtures were obtained by dry intergrinding or by mixing in the presence of a non-aqueous liquid. The only instance where rapid reaction to HAp occurred was when $CaHPO_4 \cdot 2H_2O$ and a calcium source comprising $Ca_4(PO_4)_2O$ were mixed in a non-aqueous fluid. Rapid reaction occurred regardless of whether they were mixed in the proportion needed to produce calcium deficient HAp (reaction 1 above) or to make stoichiometric HAp (reaction 3 above). Rapid reaction resulted in the formation of hydroxyapatite in 1 hour or less at 38°C. In the other cases, complete reaction was not achieved within 24 hours. The results appear in the Table below.

TABLE

FORMATION OF HAp

| EXAMPLES | PARTICLE REACTANTS | DRY INTERGRINDING WITHOUT Non-aqueous LIQUID | MIXING WITH NON-REACTING LIQUID | COMPLETE REACTION TO HAp |
|---|---|---|---|---|
| COMPARATIVE EX 1A | $6CaHPO_4 \cdot 2H_2O + 3Ca_4(PO_4)_2O$ | X | | >24 hrs. |
| INVENTIVE EX 1B | $6CaHPO_4 \cdot 2H_2O + 3Ca_4(PO_4)_2O$ | | X | <1 hr. |
| COMPARATIVE EX 2A | $6CaHPO_4 + 3Ca_4(PO_4)_2O$ | X | | >24 hrs. |
| COMPARATIVE EX 2B | $6CaHPO_4 + 3Ca_4(PO_4)_2O$ | | X | >24 hrs. |
| COMPARATIVE EX 3A | $2CaHPO_4 \cdot 2H_2O + 2Ca_4(PO_4)_2O$ | X | | >24 hrs. |
| INVENTIVE EX 3B | $2CaHPO_4 \cdot 2H_2O + 2Ca_4(PO_4)_2O$ | | X | <1 hr. |
| COMPARATIVE EX 4A | $2CaHPO_4 + 2Ca_4(PO_4)_2O$ | X | | >24 hrs. |
| COMPARATIVE EX 4B | $2CaHPO_4 + 2Ca_4(PO_4)_2O$ | | X | >24 hrs. |

WHAT IS CLAIMED IS:

1. A dry particulate agglomerate composition capable of producing phase-pure hydroxyapatite without additional sources of calcium and/or phosphate in the presence of an aqueous liquid consisting essentially of agglomerates, each individual agglomerate being a homogenous mixture of (i) $CaHPO_4 \cdot 2H_2O$ particles and (ii) particles of a calcium source comprising $Ca_4(PO_4)_2O$, said $CaHPO_4 \cdot 2H_2O$ and said calcium source comprising $Ca_4(PO_4)_2O$ being present in an amount sufficient to form phase-pure HAp having a Ca/P ratio of 1.5 to 1.67.

2. An aqueous composition which comprises:

i.) as a solids component

(A) a dry particulate agglomerate composition which consists essentially of agglomerates, each of said agglomerates being a homogenous mixture of $CaHPO_4 \cdot 2H_2O$ particles and particles of a calcium source comprising $Ca_4(PO_4)_2O$, said agglomerate being prepared by mixing (a) $CaHPO_4 \cdot 2H_2O$ particles with (b) particles of a calcium source comprising $Ca_4(PO_4)_2O$ in (c) a non-aqueous liquid, and removing said non-aqueous liquid; said $CaHPO_4 \cdot 2H_2O$ and said calcium source comprising $Ca_4(PO_4)_2O$ being present in an amount sufficient to form phase-pure hydroxyapatite having a Ca/P ratio of from 1.5 to 1.67; and

(B) a polymeric material which is capable of promoting mineralization of hydroxyapatite from said particles (A) and wherein the weight ratio of (A)/(B) is from 100/0 to 40/60, respectively: and

ii.) a physiologically acceptable aqueous liquid;

said aqueous composition having a liquid-to-total solids weight ratio of 0.15 to 1.5:

and wherein said aqueous composition reacts to form phase pure hydroxyapatite from component (A) at physiological temperatures within 1 hour.

3. The aqueous composition according to claim 2, wherein said polymeric material capable of promoting mineralization of hydroxyapatite is insoluble collagen.

4. The aqueous composition according to claim 3, wherein said insoluble collagen has been treated to form a microstructure network surface and which provides a porous structure for mineralization of said hydroxyapatite, said treated collagen having pore sizes in the range of from 2μm to 100 μm.

5. The aqueous composition according to Claim 2, further comprising from 1 to 7% by weight of sodium ions, carbonate ions, or sodium and carbonate ions based on the weight of said dry particulate agglomerate composition.

6. A method of preparing phase pure hydroxyapatite composition within 1 hour which comprises mixing solids component i) according to claim 2, with a physiologically acceptable aqueous liquid in a liquid-to-total solids weight ratio of 0.15 to 1.5; and allowing said solids component to react to form said phase pure hydroxyapatite composition within 1 hour.

7. A method of producing a particule agglomerate composition capable of producing phase-pure hydroxyapatite without additional sources of calcium and/or phosphate in the presence of an aqueous liquid, and which, optionally, can be premixed with a polymeric material capable of promoting mineralization of hydroxyapatite prior to use in a body which comprises:

(a) mixing (i) $CaHPO_4 \cdot 2H_2O$ particles with (ii) particles of a calcium source comprising $Ca_4(PO_4)_2O$ in a non-aqueous liquid, said $CaHPO_4 \cdot 2H_2O$ and said calcium

source comprising $Ca_4(PO_4)_2O$ being present in an amount sufficient to form phase-pure hydroxyapatite having a Ca/P ratio of 1.5 to 1.67, to from said particulate agglomerates; and

(b) collecting said particulate agglomerates.

8. The method according to claim 7, wherein said particles of the calcium source comprising $Ca_4(PO_4)_2O$ have a particle size of from 1 μm to 5 μm.

9. The method according to claim 7, wherein said $CaHPO_4 \cdot 2H_2O$ particles have a particle size of from 1 μm to 5 μm.

10. The method according to claim 7, wherein said non-aqueous liquid is selected from the group consisting of substituted or unsubstituted liquid aliphatic hydrocarbons and liquid aromatic hydrocarbons.

11. The method according to claim 7, wherein said collecting step (b) comprises evaporating off said non-aqueous liquid.

12. A dry particulate agglomerate composition prepared according to the method of claim 7.

13. A synthetic bone-like substitute kit comprising:

(a) a container comprising a dry particulate agglomerate composition capable of producing phase-pure hydroxyapatite without additional sources of calcium and/or phosphate in the presence of an aqueous liquid consisting essentially of agglomerates, each individual agglomerate being a homogenous mixture of (i) $CaHPO_4 \cdot 2H_2O$ particles and (ii) particles of a calcium source comprising $Ca_4(PO_4)_2O$ wherein said $CaHPO_4 \cdot 2H_2O$ and said

calcium source comprising $Ca_4(PO_4)_2O$ are present in an amount sufficient to form phase-pure HAp having a Ca/P ratio of 1.5 to 1.67.

14. The kit according to claim 13, further comprising a container of a polymeric material capable of promoting mineralization of hydroxyapatite.

15. The kit according to claim 13, wherein said polymeric material is treated insoluble collagen having a pore size of from 2 to 100 μm.

16. The composition of claim 2 further comprising up to 65% by weight of a filler, based on the weight of the solids component.

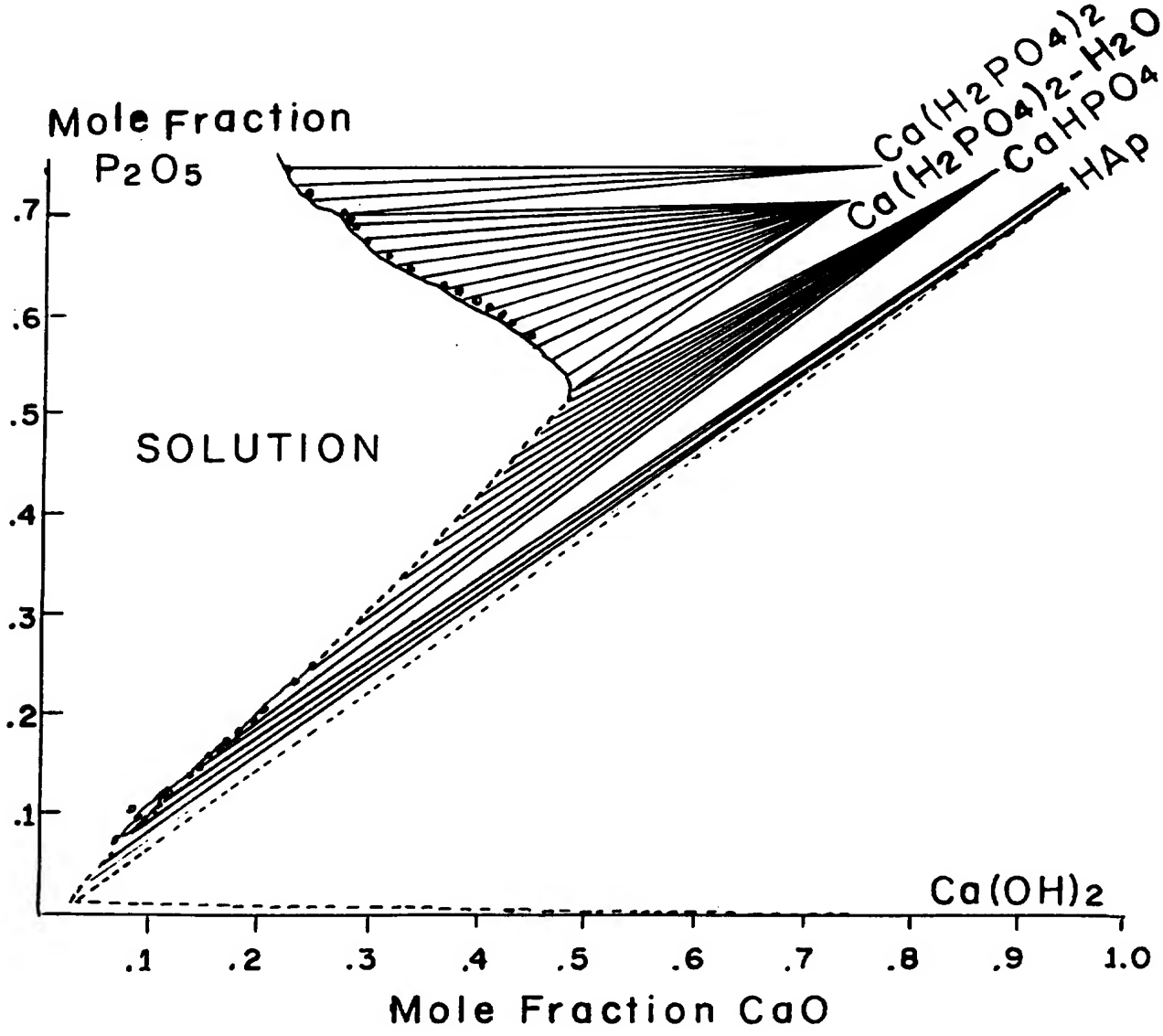
Mole Fraction
P2O5
.7
.6
.5
.4
.3
.2
.1
SOLUTION
Ca(H2PO4)2
Ca(H2PO4)2-H2O
CaHPO4
HAp
Ca(OH)2
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
Mole Fraction CaO

FIGURE 1

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03921

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :B01J 13/00; A61F 2/02, 2/28; C08K 3/32
US CL :252/313.1; 424/602, 57; 623/16; 423/308
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 252/313.1; 424/602, 57; 623/16; 423/308

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS ONLINE
Search terms: hydroxyapatite#, butane, pentane, hexane, heptane, chloroform, carbon tetrachloride, alkane#, alkene#

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y,P | US, A, 5,496,399 (ISON ET AL.) 05 March 1996, see entire document and in particular columns 3-5 and 7. | 1-16 |
| Y | US, A, 5,178,845 (CONSTANTZ ET AL.) 12 January 1993, see column 2, lines 45-54; column 3, lines 6 et sequa; column 4, lines 16 et sequa; column 5, lines 13-29; column 7, lines 13-21. | 1-16 |
| Y | US, A, 4,776,890 (CHU) 11 October 1988, see entire document and in particular column 2, lines 46 et sequa; column 3, lines 27 et sequa; column 4 and 5. | 3, 4, & 15 |
| Y | US, A, 4,612,053 (BROWN ET AL.) 16 September 1986, see entire document. | 1-16 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>22 JUNE 1996 | Date of mailing of the international search report<br>09 JUL 1996 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>DANIEL S. METZMAIER<br>Telephone No. (703) 308-0196 |

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International application No.
PCT/US96/03921

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US, A, 4,516,276 (MITTELMEIER ET AL.) 14 May 1985, see entire document and in particular column 3, lines 13 et sequa and claims. | 3, 4, & 15 |
| Y | Chem. Abstr., Vol. 111, issued November 1989 (Columbus, OH, USA), the abstract No. 179841k, Sugawara, A. 'Hydroapatite-forming compositions with improved storage stability.' JP 01-111,762 (28 April 1989), see entire abstract. | 1-2, 6-10, & 12 |